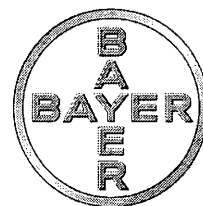


Bayer HealthCare
Bayer Schering Pharma



Department: **GDD-GED Toxicology**

GLP Report

Report No.: **AT06115**

Test Item: **PES Vorstufe 2342**

Title: **Acute Eye Irritation on Rabbits**

Study No.: **T 3081347**

Author(s): **C. Gmelin**

Study Completion Date: **November 12, 2010**

Performing Laboratory
Bayer Schering Pharma AG
GDD-GED Toxicology
42096 Wuppertal
Germany

Sponsor
Bayer MaterialScience AG

51368 Leverkusen
Germany

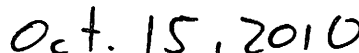
GLP Compliance Statement

The study was conducted in compliance with the principles of Good Laboratory Practice described in the following issues:

- OECD Principles of Good Laboratory Practice (as revised in 1997)
[ENV/MC/CHEM(98)17]
- Bulletin of the revised form of the chemicals act of July 2, 2008, Federal Law Gazette Volume 2008 Part I No. 28, section 6, §19, issued at Bonn July 11, 2008)



C. Gmelin
(Study Director)



Date

Quality Assurance Statement**Study No.:** T3081347**Test Item:** PES Vorstufe 2342

On the dates given below inspections were conducted by the Quality Assurance to ensure that no deviations exist that are likely to affect the integrity of this study.

The Quality Assurance Unit monitors the conduct of each study by study-based inspections or by process-based inspections of a similar type of study if the short-term nature of a study precludes inspection while it is in progress. Routine procedures and the equipment used in the relevant laboratory areas are inspected regularly and reports are made in accordance with current SOPs.

*(study plan amendments, if any, were duly audited and reported to the Study Director and Management)

Date of Audits / Inspections	Phases Audited / Inspected		Date of Report to Study Director and Management
Sep-20-2010	Study Plan *		Sep-20-2010
Sep-22-2010	process based	Clinical Observation, Raw Data / Documentation	Sep-22-2010
Oct-26-2010	Main Report	1. Draft	Oct-26-2010
Nov-10-2010	Main Report	Final Draft	Nov-10-2010

The results of this study including the methods used have been checked on the basis of the current SOPs.

They have been correctly reported and the report reflects the raw data.

In case of a multi-site study audits at the test sites are presented in the QA Statement of the Principal Investigator's report (see appendix).

Quality Assurance Unit
Global R&D Quality, GLP-Mgmt.

Date: Nov - 10 - 2010

Signature: Ursula Turek
Ursula Turek

Signatures

Study Director: C. Gmelin Nov. 12, 2010
(C. Gmelin) Date

Senior Expert: H.-W. Vohr Nov. 12, 2010
(Prof. Dr. H.-W. Vohr) Date

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PES Vorstufe 2342

1. Summary

This study was performed to assess potential irritant effects of PES Vorstufe 2342 (purity: 100 %) on the eye of rabbits.

The results are summarized in the table below.

Table 1-1 Summary of Irritant Effects (Score)							
Animal		24 h	48 h	72 h	Mean scores	Response	Reversible (days)
1	Corneal opacity	0	0	0	0.0	-	na
	Iritis	0	0	0	0.0	-	na
	Redness conjunctivae	1	0	0	0.3	-	2
	Chemosis conjunctivae	0	0	0	0.0	-	1*
2	Corneal opacity	0	0	0	0.0	-	na
	Iritis	0	0	0	0.0	-	na
	Redness conjunctivae	2	1	0	1.0	-	3
	Chemosis conjunctivae	1	0	0	0.3	-	2
3	Corneal opacity	0	0	0	0.0	-	na
	Iritis	0	0	0	0.0	-	na
	Redness conjunctivae	1	0	0	0.3	-	2
	Chemosis conjunctivae	1	0	0	0.3	-	2
response:							
corneal opacity		: mean scores		< 2 = -, ≥ 2 < 3 = +, ≥ 3 = ++			
iritis		: mean scores		< 1 = -, ≥ 1 < 2 = +, = 2 = ++			
conjunctival redness		: mean scores		< 2.5 = -, ≥ 2.5 = +			
conjunctival oedema		: mean scores		< 2 = -, ≥ 2 = +			
na : not applicable							
* : in respect of the result 1 h post application							

Conclusion:

According to classification criteria PES Vorstufe 2342 is not irritating to eyes.

There were no systemic intolerance reactions.

2. Introduction and Guidelines

The study objective was to determine the irritant effects on eyes of albino rabbits. Information derived from this test serves to indicate the possible existence of hazards likely to arise from short-term exposure of the eye and associated mucous membranes to the test substance, and - with respect to a proper handling and use - serves to permit classification (labelling) of a product.

The method used complied with the OECD - Guideline for Testing of Chemicals No. 405 - "Acute Eye Irritation/Corrosion"; adopted: 24th April 2002 and EEC Directive No. 440/2008 Part B - Method B.5.

For reasons of animal welfare a sequential testing strategy was followed in accordance with the current version of the EEC Directive No. 440/2008 and the OECD Guideline No. 405, irrespective of the requirements of other guidelines for testing for eye irritation/corrosion in rabbits.

This testing strategy comprised a stepwise approach including the evaluation of existing human and/or animal data showing effects on the eye or the skin, the performance of a SAR evaluation for eye and skin corrosion/irritation, measurement of pH value, the evaluation of data on systemic toxicity via the dermal route, the performance of a validated in vitro test for skin corrosion (T 4081285, not corrosive), an in vitro test for skin irritation (T 3081284, not irritating) and an in vitro HET-CAM test for mucosal irritation hazard before in vivo testing for eye irritation/corrosion in rabbits.

The in vitro HET-CAM test for mucosal irritation hazard is archived under T 2081283. The test compound is not irritating.

3. General Information

The study was sponsored by Bayer MaterialScience AG, 51368 Leverkusen, Germany.

The study was performed at Bayer Schering Pharma AG, GDD-GED Toxicology, 42096 Wuppertal, Germany.

3.1 Key Study Data

Study No.:	T 3081347
Study initiation date:	2010-09-16
Experimental starting date:	2010-09-21
Experimental completion date:	2010-09-24
Study completion date:	see signature page

3.2 Archiving

The study protocol, raw data and final report are retained in the archives specified by the test facility Toxicology of the Bayer Schering Pharma AG in Wuppertal. A retention sample of the test item was stored in the archive of the test facility.

3.3 Responsibilities

Study Director:	C. Gmelin
Senior Expert:	Prof. Dr. H.-W. Vohr
Test Facility Management:	Dr. T. Steger-Hartmann
Head of Test Facility:	Dr. F.-W. Jekat
Archiving:	R. Zils
Head of Quality Assurance Unit:	Dr. A. Paeßens

4. Material and Methods

4.1 Test Item

Test item:	PES Vorstufe 2342
Synonym(s):	Ester Rizinus + Sojaoel-Umesterung
EC No.:	919-697-6
Chemical name:	Castor Oil, reaction product with Soybean Oil
Batch no.:	LB06603520
Appearance:	colorless liquid
Content of test item*:	100 % (dosing is based on the test item)
Storage*:	refrigerator, amber bottle
Expiry date:	2010-10-22

*due to product information given by the sponsor

The documentation of the chemical composition of the test item is in the responsibility of the sponsor. Confirmation of the identity of the test item was performed.

4.2 Experimental Animals

The study was performed in female albino rabbits (strain Crl:KBL(NZW)BR, Charles River, 88353 Kißlegg, Germany) recommended as the preferred species for this type of studies.

Healthy adult albino rabbits free of clinical signs were used. The health of the animals was routinely examined for the main specific pathogens by the breeder. Before the study the rabbits had been vaccinated against RHD (rabbit haemorrhagic disease). No treatment with antibiotics was performed prior to receipt of the animals, or during the acclimatization phase or study period. In some cases the animals had already been used in earlier studies. This has no influence to the results of this study. Females were nulliparous and not pregnant. The acclimatization time was at least 5 days.

Body weights at start of study:

2.6 kg – 2.8 kg

Age: young adult animals

The animals were identified by labels on the cages stating at least study number, test compound, animal number and by tattooed number assigned by the breeder.

4.2.1 Husbandry and Nutrition

The animals were housed individually in cage units Metall/Noryl by EBECO. Excrement trays below the cages contained low dust wood granulate bedding (J. Rettenmaier & Söhne, 73494 Rosenberg, Germany). The wood granulate was changed at least twice weekly. The animals were regularly transferred to clean cages.

The animal room had a standardized climate:

Room temperature: $20 \pm 3^{\circ}\text{C}$

Air humidity: $50 \pm 25 \%$

Light/ Dark cycle: 12 hours rhythm.

The humidity and air temperature were continuously recorded. Occasionally deviations from the standards occurred, e.g. during cleaning of the animal room or effects of the weather. They did not have any apparent influence on the outcome of the study. The animal room was provided with sound from a radio program.

The animals received the standard diet "Ssniff K-Z" 4mm (manufacturer: Ssniff Spezialdiäten GmbH, 59494 Soest, Germany), approximately 100 g per animal per day and tap water ad libitum from polycarbonate bottles. To satisfy the needs of roughage, hay was offered additionally (hay, irradiated, delivered by Harlan Nederland, 5961 NM Horst, Netherland respectively hay pellets, manufacturer: ssniff Spezialdiäten GmbH, 59494 Soest, Germany).

The nutritive composition and the contaminant content of the standard diet were checked and analyzed routinely in random samples. No unwanted ingredients were detected. The tap water was of drinking water quality (according to the Drinking Water Decree in the current version) and analyzed routinely. The wood granulate was randomly checked for contaminants at regular intervals.

The results of these analyses have been stored at Bayer Schering Pharma AG, 42096 Wuppertal, Germany. The available data yielded no evidence of any adverse effects on the aim of the study.

The animal room was cleaned at least once a week and disinfected at least once a month. It was ensured that the diet was not contaminated, and that there was no contact of the cleaning or disinfecting solution with the test animals.

4.2.2 Number of Animals and Dose Levels

Three animals were used for the study.

4.3 Exposure Procedure

On the day before the test, both eyes of each animal were examined including fluorescein examination. Only animals with healthy intact eyes were used.

0.1 ml of the pure liquid test substance was placed into the conjunctival sac of one eye of the first animal after having gently pulled the lower lid away from the eyeball. The lids were gently held together for about one second in order to prevent loss of the test compound. The other eye, which remained untreated, served as control. The treated eye was rinsed with 0.9 % saline solution approximately 24 hours following instillation.

Because of the fact that one hour after treatment a severe irritation was not observed, a further two rabbits were treated as described.

4.4 Observations and Scoring

Eye irritations were scored and recorded approximately at 1, 24, 48 and 72 hours post application (in the following p.a.). If no irritation indices were observed after 72 h, the study was finished. If eye irritations were observed, animals were monitored usually on day 7, 14 and 21 after application until the changes had completely subsided, however for not more than 21 days after application.

The degree of ocular lesions was recorded as specified by DRAIZE (see Annex page 20) and any serious lesions or toxic effects other than ocular lesions were also recorded and fully described.

As general criteria the body weight of each animal was recorded at the beginning of the study. If clinical findings other than eye irritations occur they were recorded daily.

4.5 Evaluation and Interpretation of Results

The interpretation of the results is based on the EEC Directive No. 440/2008 (see Annex page 21).

Table 5-1 Irritant Effects on the Eye

- : no further examination

Table 5-2 Summary of Irritant Effects (Score)

Animal		24 h	48 h	72 h	Mean scores	Response	Reversible (days)
1	Corneal opacity	0	0	0	0.0	-	na
	Iritis	0	0	0	0.0	-	na
	Redness conjunctivae	1	0	0	0.3	-	2
	Chemosis conjunctivae	0	0	0	0.0	-	1*
2	Corneal opacity	0	0	0	0.0	-	na
	Iritis	0	0	0	0.0	-	na
	Redness conjunctivae	2	1	0	1.0	-	3
	Chemosis conjunctivae	1	0	0	0.3	-	2
3	Corneal opacity	0	0	0	0.0	-	na
	Iritis	0	0	0	0.0	-	na
	Redness conjunctivae	1	0	0	0.3	-	2
	Chemosis conjunctivae	1	0	0	0.3	-	2

response:

corneal opacity : mean scores < 2 = -, ≥ 2 < 3 = +, ≥ 3 = ++

iritis : mean scores < 1 = -, ≥ 1 < 2 = +, = 2 = ++

conjunctival redness : mean scores < 2.5 = -, ≥ 2.5 = +

conjunctival oedema : mean scores < 2 = -, ≥ 2 = +

na : not applicable

* : in respect of the result 1 h post application

6. Conclusion

The irritant / corrosive effects of PES Vorstufe 2342 were tested on the eye of rabbits. The method used complied with the OECD - Guideline for Testing of Chemicals No. 405 - "Acute Eye Irritation/Corrosion"; adopted: 24th April 2002 and EEC Directive No. 440/2008 Part B - Method B.5.

According to classification criteria PES Vorstufe 2342 is not irritating to eyes.

There were no systemic intolerance reactions.



Ministerium für Arbeit, Gesundheit und Soziales
Des Landes Nordrhein-Westfalen

Pfaffenwall 25, 40219 Düsseldorf

Aktenzeichen II A 5 – 31.11.46.06

Gute Laborpraxis/Good Laboratory Practice
GLP-Bescheinigung/Statement of GLP Compliance
(gemäß/according to § 19b Abs. 1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung Assessment of conformity with GLP according to
der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Chemikaliengesetz and Directive 88/320/EEC at:
Richtlinie 88/320/EG wurde durchgeführt in:

☒ Prüfeinrichtung/Test facility

☐ Prüfstandort/Test site

Bayer HealthCare AG

BSP-GDD-GED

Toxikologie

Aprather Weg 18 a

42096 Wuppertal

Prüfungen nach Kategorien

(gemäß ChemVwV-GLP Nr. 5.3/OECD guidance)

Areas of Expertise

(according ChemVwV GLP Nr. 5.3/OECD guidance)

Kategorie 1

Prüfungen zur Bestimmung der
physikalisch-chemischen Eigenschaften
und Gehaltsbestimmungen

category 1

physical-chemical testing

Kategorie 2

Prüfungen zur Bestimmung der
toxikologischen Eigenschaften

category 2

toxicity studies

Kategorie 3

Prüfungen zur Bestimmung der
erbgutverändernden Eigenschaften (in
vitro und in vivo)

category 3

mutagenicity studies

Kategorie 9

Biochemische Toxikologie;
Kurzzeitkanzerogenese;
Immuntoxikologie;
Sicherheitspharmakologie

category 9

biochemical toxicology;
short time cancerogenicity;
immunotoxicity;
safety pharmacology

Datum der Inspektion

01.Sept.2008 bis 05.Sept.2008

Date of Inspection

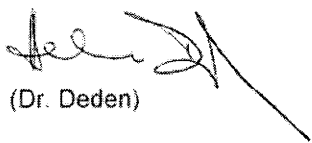
September 1st 2008 until September 5th 2008

Die/Der genannte Prüfeinrichtung/Prüfstandort befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung/diesem Prüfstandort die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.

Düsseldorf, den 09.02.2009

Im Auftrag


(Dr. Deden)

The above mentioned test facility/ test site is included in the national GLP Compliance Programme and is inspected on a regular basis.

Based on the inspection report it can be confirmed, that this test facility/test site is able to conduct the aforementioned studies in compliance with the Principles of GLP.



Dienstsiegel/official-seal

Grading of Ocular Lesions

Cornea*

Opacity: degree of density (readings should be taken from most dense area)	
No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal lustre); details of iris clearly visible	1
Easily discernible translucent area; details of iris slightly obscured	2
Nacrous area; no details of iris visible; size of pupil barely discernible	3
Opaque cornea; iris not discernible through the opacity	4
Maximum possible: 4	

* The area of corneal opacity should be noted

Area of Corneal Opacity

More than 0, but less than $\frac{1}{4}$	1
More than $\frac{1}{4}$, but less than $\frac{1}{2}$	2
More than $\frac{1}{2}$, but less than $\frac{3}{4}$	3
More than $\frac{3}{4}$	4
Maximum possible: 4	

Iris

Normal	0
Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia, or injection; iris reactive to light (a sluggish reaction is considered to be an effect)	1
Hemorrhage, gross destruction, or no reaction to light	2
Maximum possible: 2	

Conjunctivae

Redness (refers to palpebral and bulbar conjunctivae; excluding cornea and iris)	
Normal	0
Some blood vessels hyperaemic (injected)	1
Diffuse, crimson color; individual vessels not easily discernible	2
Diffuse beefy red	3
Maximum possible: 3	

Chemosis

Swelling (refers to lids and/or nictating membranes)	
Normal	0
Some swelling above normal	1
Obvious swelling, with partial eversion of lids	2
Swelling, with lids about half closed	3
Swelling, with lids more than half closed	4
Maximum possible: 4	

Interpretation of Results

Serious damage to eyes

An eye irritant with risk of serious damage to eyes is a test material that produces:

- effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days
and/or
- irreversible colouration of the eyes
and/or
- at least in 2 of 3 tested animals, a positive response of:
 - corneal opacity* ≥ 3
and/or
 - iritis* = 2

Irritating to eyes

An eye irritant with risk of significant ocular lesions to eyes is a test material that produces any of the values:

- at least in 2 of 3 tested animals, with a positive response
(fully reversed within an observation period of normally 21 days) of:
- corneal opacity* ≥ 2 but < 3
and/or
 - iritis* ≥ 1 but < 2
and/or
 - conjunctival redness* ≥ 2.5
and/or
 - conjunctival oedema* ≥ 2

*: calculated as the mean scores at the reading times 24, 48 and 72 hours.
